Control of Seasonal Development by Photoperiod and Temperature in the Linden Bug, *Pyrrhocoris apterus* in Belgorod, Russia

AIDA H. SAULICH[1], TATYANA A. VOLKOVICH[1] and HIDEHARU NUMATA[2]*

[1]*Laboratory of Entomology, Biological Research Institute, St. Petersburg University, St. Petersburg 198904, Russia* [2]*Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi, Osaka 558, Japan*

ABSTRACT—The life cycle of the Belgorod population of the linden bug, *Pyrrhocoris apterus* (L.) (Heteroptera, Pyrrhocoridae) was observed in the field, and under quasi-natural rearing conditions. There were univoltine and bivoltine pathways in the life cycle. The emergence of diapause adults under quasi-natural conditions was consistent with the parameters of photoperiodic response obtained by experiments at constant photoperiod and temperatures. The temperature dependence of the photoperiodic induction of diapause plays a significant role in life cycle adaptation in this species.

INTRODUCTION

It is accepted that photoperiodic responses play a major role in the control of seasonal life cycles in insects [2–4, 14]. However, it is often difficult to apply laboratory results obtained under constant photoperiods and temperatures to predict seasonal development in the field. Therefore, phenological observation in the field and experiments under natural conditions are better ways to discuss life cycle adaptation in an insect.

The linden bug, *Pyrrhocoris apterus*, has an adult diapause controlled by a long-day photoperiodic response [5, 9, 12, 16]. In a previous paper, we showed the photoperiodic response of *P. apterus* in Belgorod Region, Russia, under conditions of constant temperature and under thermoperiodic conditions in the laboratory. Although thermoperiod had a slight effect on the induction of diapause, the critical daylength essentially depended on the mean temperature [9]. However, the adaptive significance of this temperature dependence remained unclear.

In all populations of *P. apterus* examined until the present, the diapause is facultative [5, 9, 12, 16], and therefore they all have the possibility to produce two or more generations a year. However, a univoltine life cycle has been observed in the population in Paris, France [10], and in that in central Bohemia [5, 6]. In Kazakhstan and Ukraine, the possibility of a partially bivoltine life cycle was reported [1, 15]. Until recently, however, no reliable evidence has been shown of the voltinism in *P. apterus*, because adults of this species have a long life-span and oviposition period and therefore it is difficult to discriminate the generation of adults. Recently, Socha and Šula [13] showed the occurrence of two generations of *P. apterus* in one year in southern Bohemia.

In the present study, we first observed the life cycle of the Belgorod population of *P. apterus*, and then reared the insects under quasi-natural conditions. We discuss a likely role for temperature dependency of the photoperiodic response in the life cycle adaptation of *P. apterus* based on a comparison of the laboratory findings [9] with those under quasi-natural conditions.

MATERIALS AND METHODS

Observations and experiments were carried out in the reservation, "Forest on the River Vorskla" (50°N, 36°E), in Belgorod Region, Russia, on the local population of *P. apterus* in 1990 and 1991.

Nymphs of *P. apterus* were reared from the first instar in a meteorological booth placed in the open, by the methods previously described [9]. The shelf where insects were reared was about 130 cm high from the ground. Only the north side of the booth opened and the insects inside were protected from direct sunlight. The temperature in the booth was recorded. After adult emergence, the insects were reared as male and female pairs in Petri dishes (9 cm diameter). Oviposition was recorded daily, and if a female did not lay eggs for 30 days from emergence we judged it to be in diapause.

Fifth- (final-) instar nymphs were collected from the field and raised to adults in cages (45 cm diameter, 80 cm height) on the soil surface. The cage was made of a wire-frame with a gauze net covering the top and sides, and the frame was buried into the soil at a depth of 10 cm. Thick branches and pieces of bark were placed in the cage to protect the insects from rain and direct sunlight. The temperature on the soil surface in the cage was recorded. The incidence of diapause was examined as for the insects in the booth.

Some insects were reared from the first instar in the laboratory under 18L-6D at 25±1°C, and transferred to 16L-8D or 12L-12D during the fifth instar. Their diapause status was examined as stated above.

RESULTS

Phenological observation

Adults after overwintering appeared on the ground in late April both in 1990 and in 1991. On warm sunny days from early May, we observed mating and oviposition. Thereafter we found both nymphs and adults until late autumn. Because adults lived and continued to lay eggs for a long period, it was difficult to divide generations only by field observations. We could only determine the beginning of emergence of new adults, because they had a richer red color and softer integument than older adults. These differences disappeared a few days after adult emergence. In 1990, we observed new adults at the beginning of July,

although we did not record the date of their earliest emergence. In 1991, new adults emerged from mid-June.

Rearing from the first instar

To define the number of generations in a year, we reared *P. apterus* from the first instar in a meteorological booth under quasi-natural conditions in 1990 and 1991. We used eggs laid by adults after overwintering. In 1990, we placed first-instar nymphs just after hatching in the booth on 3, 13 and 20 June. In this year, it was cool in June; the mean temperature for ten days was 13.6–15.5°C. Therefore, the nymphs developed slowly and new adults first emerged as late as 7 August. All adults entered diapause except for only one that emerged on 10 August (Fig. 1).
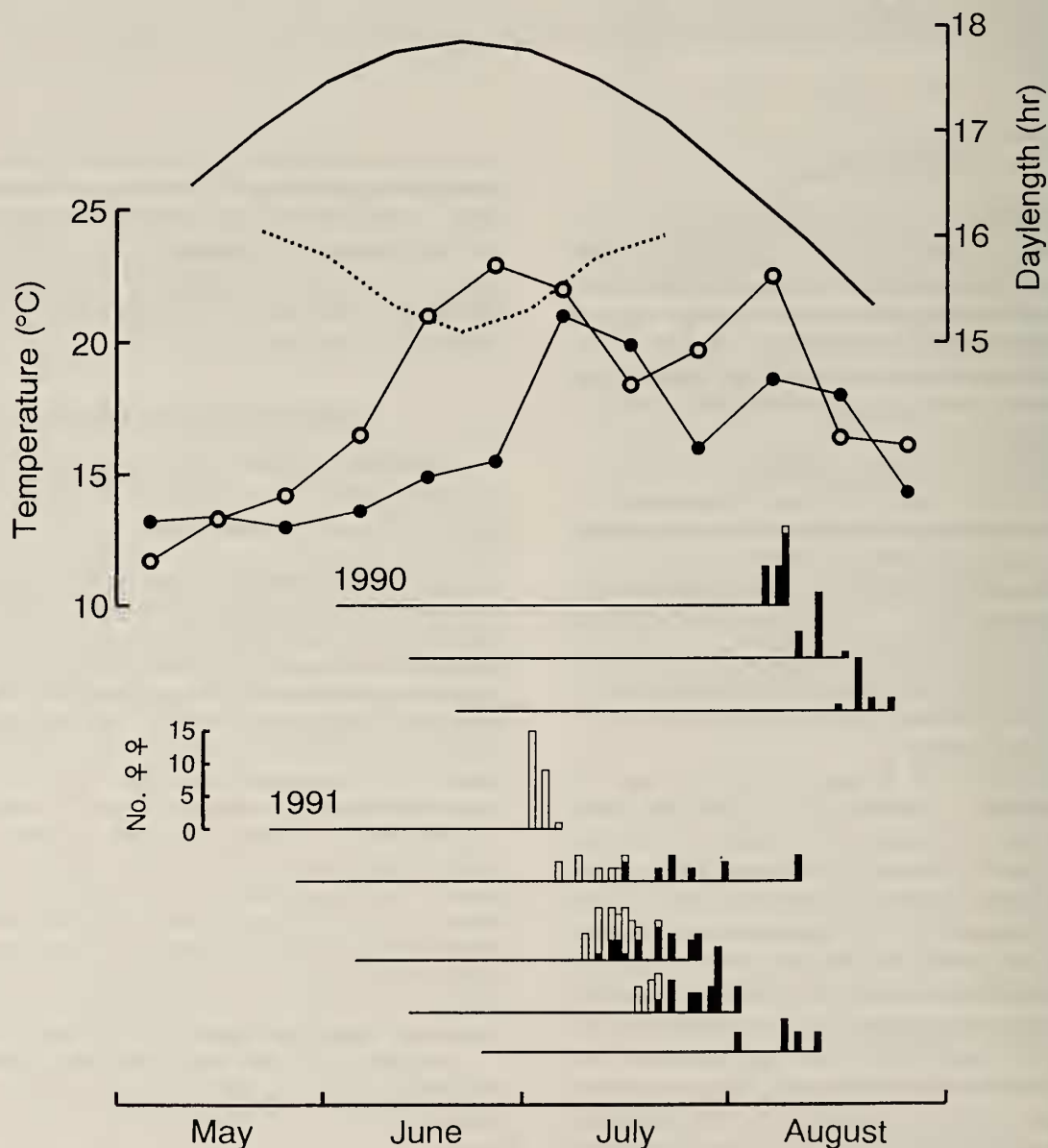

FIG. 1. Induction of adult diapause in *Pyrrhocoris apterus* under quasi-natural conditions in 1990 and 1991. Each horizontal line shows an experimental series. The outset corresponds to the hatching of the nymphs. Date of adult emergence in females is shown at the end of line as histograms. Black, diapause; white, reproductive. Circles show the mean temperatures for ten days in the meteorological booth used for the experiments in 1990 (closed) and in 1991 (open). Solid line, natural daylength including civil twilight at 50°N (after Beck [2]). Dotted line, critical temperature for the induction of diapause in *P. apterus* (after Numata *et al.* [9]) (see Fig. 2).

In 1991, we placed first-instar nymphs in the booth on 23 and 27 May, 6, 13 and 25 June. In this year, the mean temperature for ten days in June was 16.5–22.9°C, and therefore nymphs developed much faster than in 1990. New adults first emerged on 2 July. All females emerging before 10 July laid eggs. From 12 to 21 July, both reproductive and diapause female adults emerged, and all females emerging after 22 July entered diapause (Fig. 1).

Rearing of the field-collected nymphs

Thus, we knew when diapause adults emerged in the booth under quasi-natural conditions. However, the temperature in the booth used in this experiment differed from that on the soil surface where the insects develop in nature. Furthermore, the nymphs of this species behaviorally select microhabitat [8]. Therefore, to determine the actual time of emergence of diapause adults, we collected fifth-instar nymphs from the field and kept them in cages on the soil surface in 1991. After adult emergence, we examined their diapause status (Table 1). From the samples collected on 7 June, adults emerged on 9–14 June, which corresponded to the first emergence of new adults in nature. They were all nondiapause adults and began to lay eggs in late June. On 21 July, the first diapause adults emerged, and thereafter diapause adults prevailed in the samples. In some periods there was only a little difference between the temperature on the soil surface and that in the meteorological booth, although in other periods the former was much higher than the latter. For example, in late April the mean temperature of the soil surface was 23.3°C and that in the booth was 10.3°C, while in mid- and late July, the difference was 3.6–3.8°C.

TABLE 1. Incidence of adult diapause in *Pyrrhocoris apterus* collected as fifth-instar nymphs from the field in 1991

Date of collection	No. of nymphs collected	Date of adult emergence	No. of adult pairs	% diapause
7 June	46	9–14 June	20	0
2 July	35	4–6 July	22	0
12 July	32	14–18 July	18	0
		21 July	6	33
22 July	70	30 July–2 August	19	84
		6 August	15	75

Photoperiodic sensitivity

In *P. apterus*, the photoperiodic sensitivity commences in the fourth or fifth instar and continues throughout the adult life [5]. Five or six short-day cycles experienced before adult emergence were enough to induce diapause in the population from central Bohemia [7]. We examined the photoperiodic sensitivity in the Belgorod population at 25°C. We used 18L-6D as long-day conditions, and 16L-8D or 12L-12D as short-day conditions, because the critical daylength was about 17 hr at 25°C [9].

Nine short-day cycles experienced before adult emerg-

TABLE 2. Effect of photoperiodic transfer from long-day to short-day conditions before adult emergence on the induction of adult diapause in *Pyrrhocoris apterus* at 25°C

Photoperiodic conditions	Photoperiodic transfer, —days before adult emergence	No. of pairs used	% diapause
18L-6D→16L-8D	12	25	100
	9	26	100
	8	20	80
	4	24	50
	0	25	8
18L-6D→12L-12D	9–11	26	100
	4	17	35
	0	26	0

ence were enough to induce adult diapause in all individuals, and four cycles were critical, irrespective of the short-day conditions used (Table 2).

DISCUSSION

Interaction between photoperiod and temperature

In the Belgorod population of *P. apterus*, the photoperiodic induction of adult diapause depends on temperature, and the critical daylength is longer at lower temperatures [9], as in many other insects with long-day photoperiodic responses [2–4, 14]. Moreover, the critical daylength under thermoperiodic conditions is close to that at the constant temperature equivalent to the mean temperature of the thermoperiod [9]. This species is slightly sensitive to a gradual increase of the photophase only in the threshold range [17]. Therefore, we examined whether we could apply the photoperiodic response obtained experimentally at constant photoperiods and temperatures to the natural induction of diapause. First, we determined the critical temperature for a given daylength, as we had ascertained the critical daylengths at each temperature (Fig. 2). Then, we showed
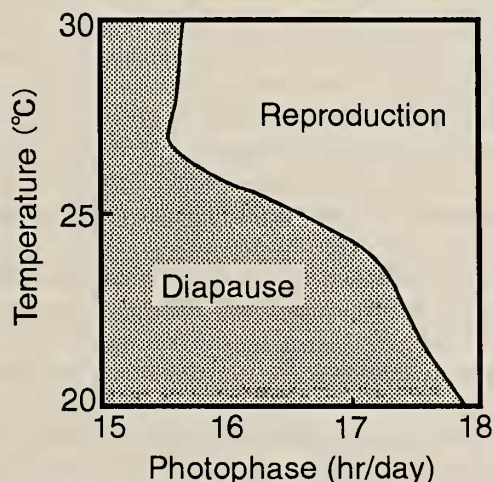

FIG. 2. Effect of photoperiod and temperature on the induction of adult diapause in *Pyrrhocoris apterus* (after Numata *et al.* [9])

the critical temperature for the natural daylength as a dotted line in Figure 1. During periods when the environmental temperature is lower than this value, diapause might be induced.

Voltinism of the Belgorod population

In the Belgorod population of *P. apterus*, there were two alternative pathways in the life cycle, i.e., univoltine and bivoltine. In 1990 (cool weather), new adults emerged from early August, and most of these entered diapause. The mean temperature for ten days never exceeded the critical value for the induction of diapause. Low temperature contributed to the induction of diapause in two ways: First, it lengthened the critical daylength for the induction of diapause. Second, it delayed nymphal development, and therefore the sensitive stage was postponed to the period when the daylength was short enough to induce diapause.

In 1991 (warm weather), however, new adults emerged from early July, and early emerging adults of the first generation reproduced. Their progeny, i.e., the second generation, developing in August and September should enter diapause. Furthermore, late emerging adults of the first generation also entered diapause. The mean temperatures for mid- and late June and early July were higher than the critical value for the induction of diapause. All adults emerging on and after 22 July entered diapause. When insects were transferred from long-day to short-day conditions nine days before adult emergence in the laboratory, all of them entered diapause. Therefore, the emergence of diapause adults under quasi-natural conditions is consistent with the parameters of photoperiodic response obtained by the experiments at constant photoperiods and temperatures.

In the regions where the duration of the growing season in individual years is nearly constant, photoperiod is an effective cue for the prediction of the coming season. Sauer *et al.* [11] regarded the temperature dependence of the critical daylength in *Pieris brassicae* both as an adaptation to the low predictability of the end of the growing season, and as an adaptation related to migration. *P. apterus* does not migrate and therefore the temperature dependence of the photoperiodic response has adaptive significance where the temperature varies much between years as in Belgorod Region.

Although Honěk and Šrámková [8] pointed out that the production of the second generation as an unfavorable modification of the life cycle in *P. apterus* in central Bohemia, there is no doubt that the Belgorod population of *P. apterus* partially produces two generations in warmer years. However, it seems that even in cooler years the Belgorod population is not homogeneously univoltine, because we found some new adults laying eggs in early July even in 1990, when it was cool (unpublished). Furthermore, the proportion of diapause adults emerging from the fifth-instar nymphs collected from the field was lower than that in insects reared from the first instar under quasi-natural conditions in 1991. We attribute these differences in part to the differences in temperature between the meteorological booth and the soil surface where *P. apterus* lives in nature, because the temperature on the soil surface was sometimes much higher than that in the meteorological booth. Furthermore, we observed in spring that nymphs and adults aggregated in warm places on the soil surface where the temperature was much higher than in the meteorological booth because of radiant heat. Therefore the behavioral regulation of body temperature may also contribute the difference between the quasi-natural and the real natural conditions. However, we cannot predict the seasonal development of *P. apterus* with the temperature on the soil surface, because during the warm seasons the nymphs of *P. apterus* select microhabitat where the temperature is much cooler than on the soil surface [8].

Retardation of nymphal development

The nymphal development of *P. apterus* is retarded at the critical daylength for the induction of adult diapause or a little shorter [9, 12]. The combination of photoperiod and temperature in early July in 1990 was in the range where nymphal development is retarded in the laboratory [9], although it is unclear whether the slow development of nymphs under quasi-natural conditions in 1990 resulted from retardation due to the threshold range or only from the normal effect of temperature. The combination of photoperiod and temperature in both mid-June and early July in 1991 was also in the range where nymphal development is retarded in the laboratory [9]. Thus, in warmer years nymphs possibly meet with threshold conditions twice throughout the season.

We assume that at threshold conditions before summer solstice, nymphs had not reached the sensitive stage for the retardation of development because no nondiapause adults had an extremely long nymphal period in the present study. Under threshold conditions after the summer solstice, prolongation of the nymphal period will delay the emergence of diapause adults, as Saunders [12] suggested in the univoltine life cycle in the population of this species in central Bohemia. In the second series in 1991 under quasi-natural conditions, some adults emerged much later than the others and entered diapause. It has been generally accepted that the range of temperature and photoperiod for the induction of winter diapause is adequate to reach the diapause stage before winter (e.g., [11]). The diapause adults developing in earlier seasons have enough time before winter and prolongation of the nymphal period is less harmful for them than for those developing in later seasons. If the diapause adults emerging after an extended nymphal period have a larger body size, higher fecundity or lower mortality in winter, the retardation of nymphal development would have adaptive significance. Further studies are needed to clarify these.

ACKNOWLEDGMENTS

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